04 – SMARCA4 REDIRECTS BINDING OF MACROPHAGE ACTIVATING TRANSCRIPTION FACTOR 1 (ATF1) FROM GENES FOR INFLAMMATION RESOLUTION TO GENES FOR ERYTHROCYTE RESOLUTION

A Seneviratne 1, L Cave 1, A Shaikh 1, D Carling 2, JC Mason 1, DO Haskard 1, JJ Boyle 1.
1 - National Heart and Lung Institute, 2 - London Institute of Medical Sciences, Imperial College London

Atherosclerosis is inflammatory. The resolution of inflammation, including atherosclerotic inflammation, is an emerging area. Genome wide association studies (GWAS) have revealed a number of risk loci, some of which are associated with the location of *SMARCA4*, a SWI/SNF chromatin remodelling gene important for gene activation. We tested whether *SMARCA4* is an independent atherosclerotic risk gene with a patho-mechanism relevant to atherosclerosis. We find that *SMARCA4* and *LDLR* are genetically independent in large scale genetic association studies. We describe an AMPK/MSK1/H3S10p pathway that modifies chromatin in response to heme. This pathway results in gene-selective H3S10 phosphorylation, seen with two classically cyclic-AMP-responsive genes, *FOS* and *NR4A2*, but not the heme response gene HMOX1. At the *HMOX1* cis-regulatory sequence, chromatin looping and recruitment of SMARCA4 occurred prior to recruitment of p-ATF1. Knockdown of SMARCA4 suppressed p-ATF1 binding to *HMOX1* but increased its binding to the cyclic-AMP response elements of the enhancers for *FOS* and *NR4A2*. This resulted in suppressed *HMOX1* mRNA levels, but increased mRNA levels for *FOS* and *NR4A2*. Downstream, si-*SMARCA4* allowed heme to induce *PLA2G7*, which encodes platelet-activating-factor acetyl hydrolase (PAF-AH). These data point to a role for SMARCA4 in chromatin remodelling in advance of ATF1, with the ability to alter its preferred target genes. Taken together, these data indicate that SMARCA4 is an independent atherosclerosis risk gene, is associated with a novel mechanism, and is involved in switching between leukocyte-resolution and erythrocyte-resolution.

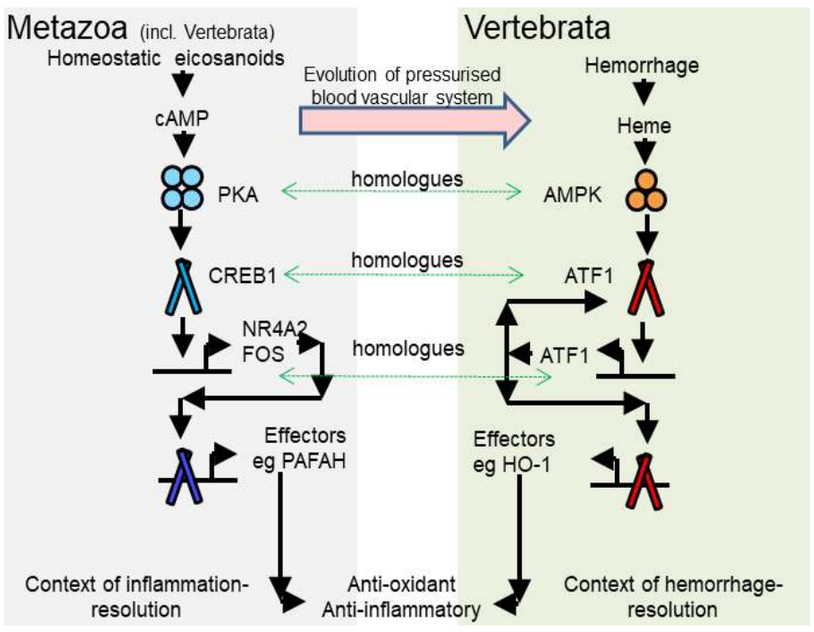


Figure 1 A, Putative evolution of ATF1-mediated hematoma resolution relative to CREB1-mediated inflammation-resolution Left grey box – older mechanism found in both invertebrates and vertebrates. Specialised pro-resolving mediators (SPMs) including resolvins activate macrophages to elevate intracellular c-AMP. This leads to a classical pathway involving CREB1-phosphorylation, 'homing' to c-AMP response elements and inducing target genes. Initially, these are FOS and NR4A2, but in turn these regulate genes that suppress inflammation such as *PLA2G7* (PAFAH). **Right pale green box** –AMPK is a homologue of PKA and is activated by heme. AMPK activates ATF1. This activates an alternative set of genes that also promote tissue homeostasis, but are more oriented towards clearance of erythrocytes. This second pathway evolved in response to tissue hemorrhages, which are likelier with the development of a pressurised vascular system.

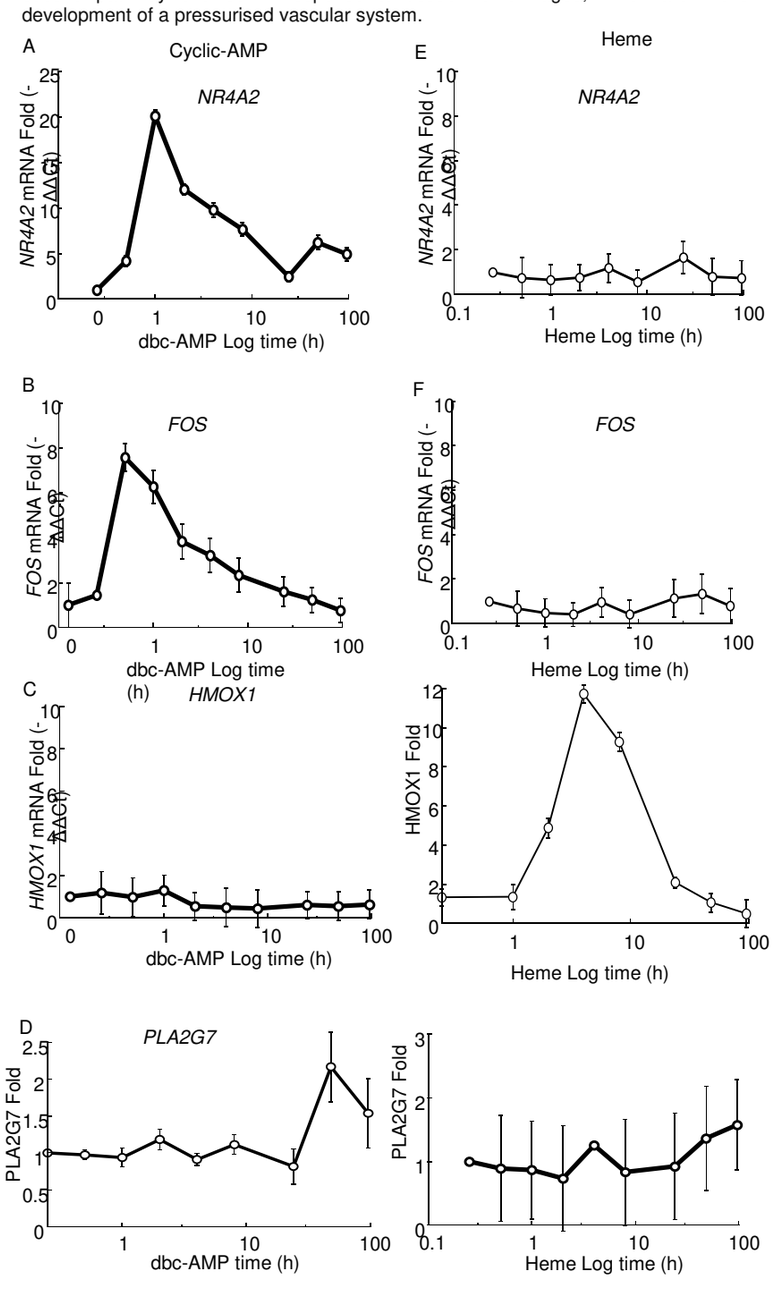


Figure 2, Specificity of heme-induced genes and c-AMP-induced genes. Also, dbc-AMP induces the anti-inflammatory gene *PLA2G7* (Lp-PLA2, PAF-AH). Genes as indicated, measurements by standard RT-qPCR Mean SE, n=12 donors

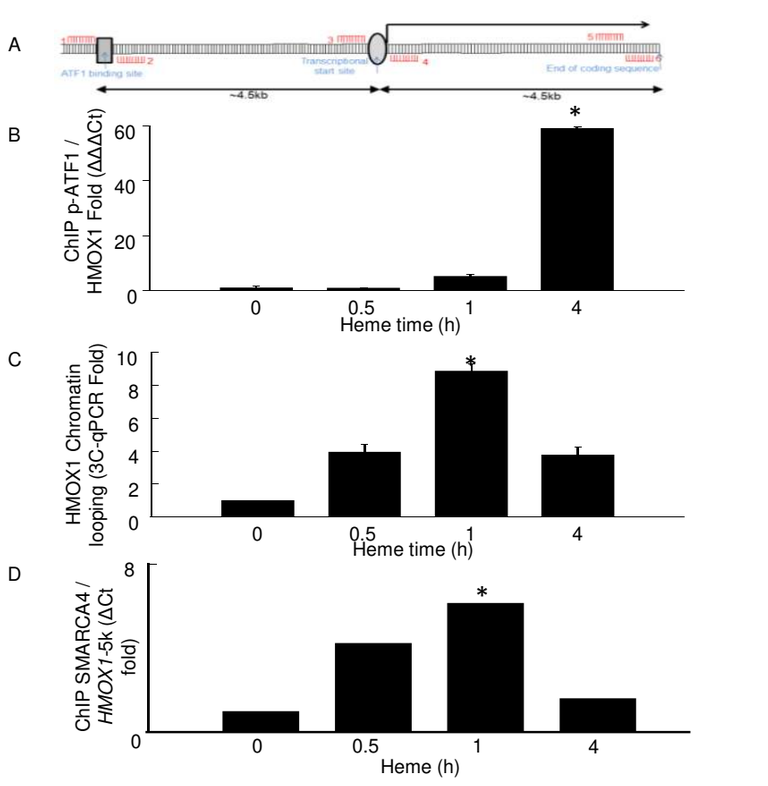


Figure 3, Enhancer remodelling precedes Transcription Factor Binding. A, Primers and sites for chromatin. Scales are approximate. ChIP – ATF1-site primers 1+2, ChIP – genomic control for normalisation primers 5+6, ChIP ΔΔCt – Ab[Ct(1,2)-Ct(5,6)]- IgG[Ct(1,2)-Ct(5,6)], 3C ΔΔCt – mean[(1,4),(2,3)]- mean[(1,2),(5,6)]. **B, ATF1 ChIP-qPCR**. Macrophages (n=4 donors) are stimulated with heme for the indicated times; then fixed (1% molecular biology formaldehyde); chromatin sheared; immunoprecipitated with p-ATF1 or IgG control and DNA measured by qPCR, using primers for the -5k CRE site of human *HMOX1*, relative to control sequence. C, Chromatin conformation capture analysis (3C) measured by qPCR. Macrophages (n=4 donors) are stimulated with heme for the indicated times; then fixed (1% molecular biology formaldehyde); restriction digested (Bgl-III); and religated with T4 Ligase at limiting dilution. DNA is purified and measured by qPCR, using primers specific for loops between the -5k CRE site of human *HMOX1*, and the -109 site in the core promoter**. D, ChIP for SMARCA4,** methods outlined above, mean, SE, n=4.

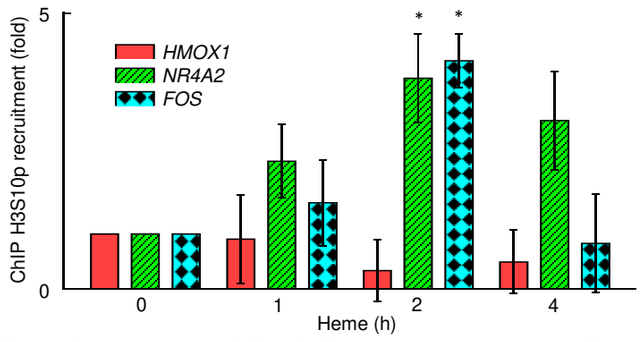


Figure 4, Chromatin Immunoprecipitation ChIP for phosphorylated Histone H3 instead, and qPCR done for HMOX1 and two c-AMP responsive genes for reference, showing signal specificity

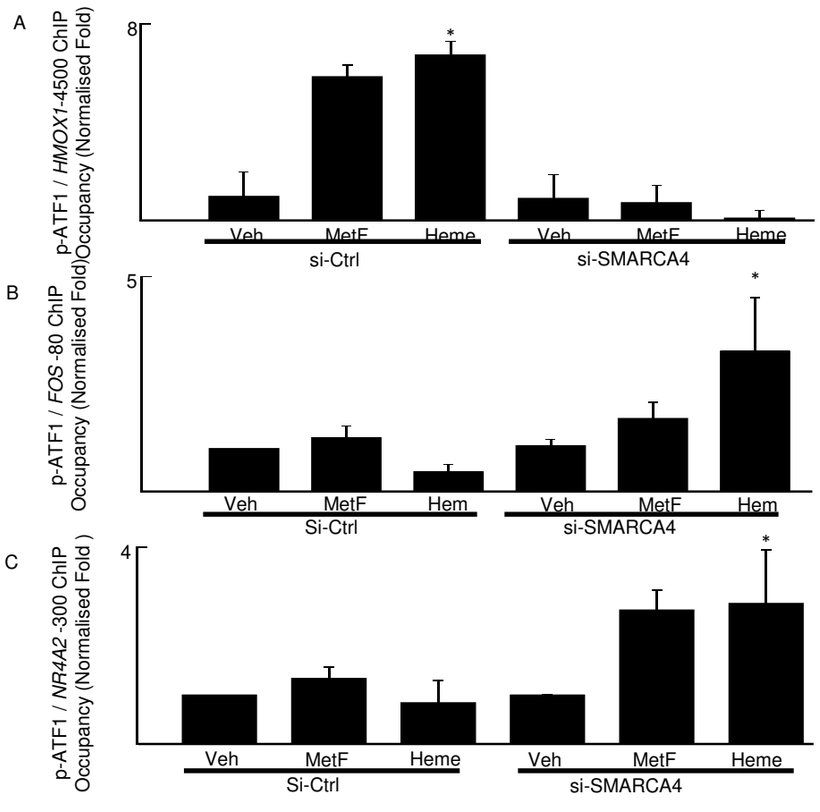


Figure 5 SMARCA4-knockdown relocates p-ATF1 from heme-targets to c-AMP targets A-C, siRNA for SMARCA4 redistributes p-ATF1 in heme-activated human monocyte-derived macrophages from signature heme-inducible genes to signature c-AMP-inducible genes. ChIP was by methods in Figure 3 and siRNA by our published methods. Metformin was used as a second activator of AMPK. Si-SMARCA4 decreases p-ATF1 recruitment to *HMOX1* (A) but increases it to *FOS* (B) and *NR4A2* (C). **D**, In hMDM, si-SMARCA4 knockdown prevents heme-induced chromatin looping at the *HMOX1* enhancer (methods as Figure 3).

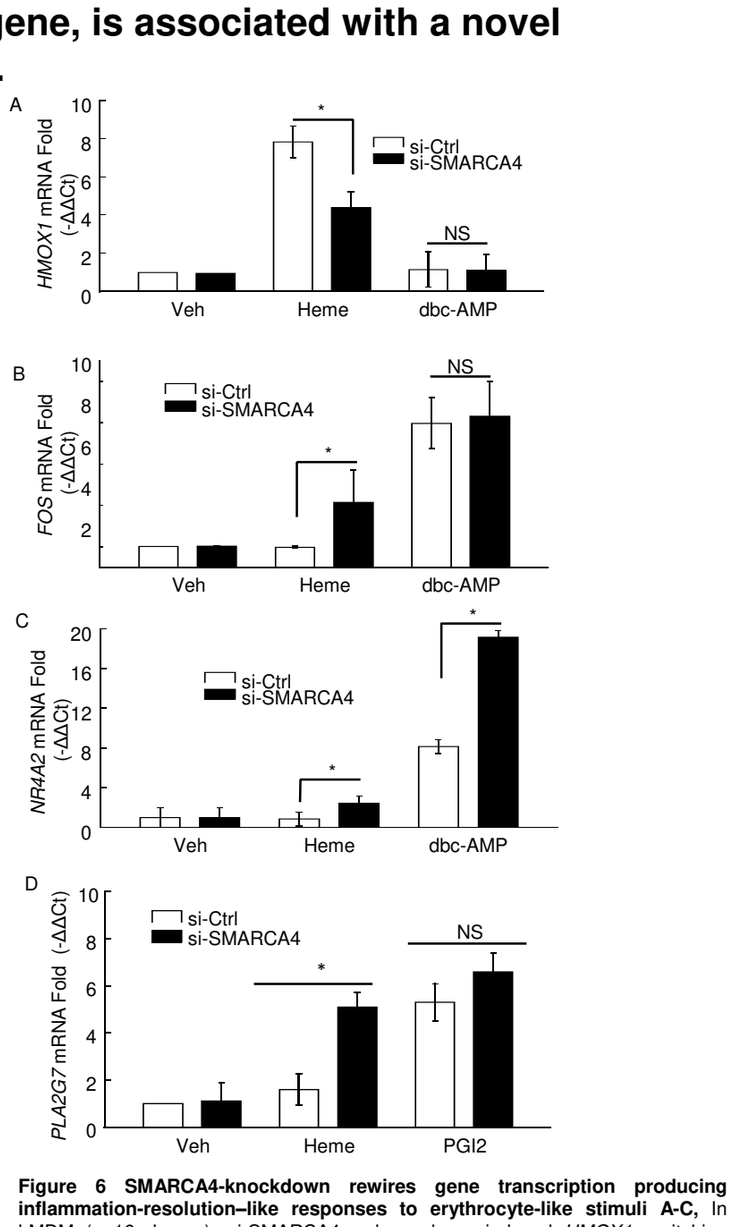
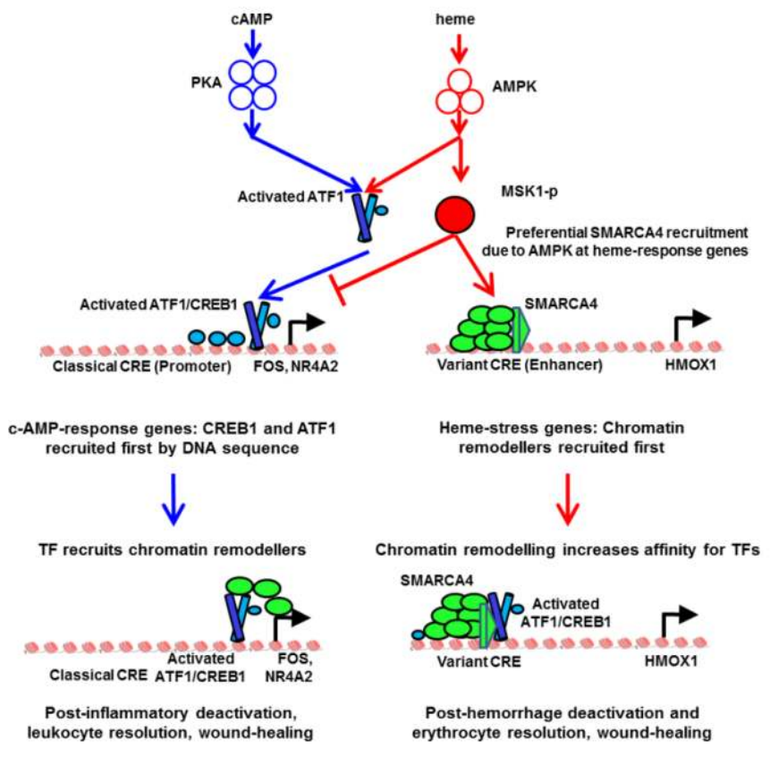


Figure 6 SMARCA4-knockdown rewires gene transcription producing inflammation-resolution–like responses to erythrocyte-like stimuli A-C, In hMDM (n=16 donors), si-SMARCA4 reduces heme-induced *HMOX1*, switching over to heme-induced *FOS* and *NR4A2*. Cyclic-AMP-induced *NR4A2* is potentiated by si-SMARCA4 (*p<0.01). This indicates that si-SMARCA4-induced redistribution of p-ATF1 produces functional differences in gene expression. Moreover, SMARCA4-knockdown is does not generally suppress gene activation. **D**, SMARCA4 (BRG1) knockdown in hMDM (n=6) switches heme to inducing *PLA2G7* like PGI_2, consistent with switching back to inflammation-resolution.



B, Contribution of SMARCA4 to specificity of signalling and gene-regulation between c-AMP and heme Left (red) pathway – canonical c-AMP mediated gene activation. Classically, c-AMP activates c-AMP-dependent kinase (PKA), which phosphorylates CREB1, which homes to canonical transcription factor binding sites in the enhancers of c-AMP response genes, such as FOS (an immediate early gene and transcription factor) and NR4A2 (an orphan nuclear receptor transcription factor). Enhancer occupancy by CREB1 recruits chromatin remodelling enzymes (eg SMARCA4) and eventually RNA-polymerase (POL2) leading to gene expression. **Right (blue) pathway – heme related gene activation at variant c-AMP response elements.** These are not normally high-enough affinity to bind p-ATF1 or p-CREB1. Heme activates the PKA-homologue AMPK. AMPK activates MSK1, which phosphorylates Histone H3. the histone H3 phosphorylation occurs differentially at heme-response genes and c-AMP-response genes leads to differential recruitment of SMARCA4. In turn, this selectively increases the affinity for p-ATF1, of CRE sites upstream of heme-response genes over those of c-AMP response genes. Once AMPK activates ATF1 by phosphorylation, it preferentially activates heme-response genes.

Imperial College London

British Heart Foundation